Alginate degrading bacteriophages as a therapeutic treatment against *Pseudomonas aeruginosa* in cystic fibrosis

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BACKGROUND

- *Pseudomonas aeruginosa* can cause up to 20% of nosocomial infections [1].
- *P. aeruginosa* colonize the lung of cystic fibrosis patients.
- *Pseudomonas* infections are difficult to control because its resistance to many antibiotics and disinfectants, mainly due to efflux pumps an biofilm [2].
- Phage therapy has many advantages over antibiotics but needs further research.
- The use of genetically-modified phages is an interesting site-directed treatment.

INITIAL HYPOTHESIS AND OBJECTIVES

- A cocktail composed by *P. aeruginosa* bacteriophages overexpressing an alginate-degrading enzyme could be an effective treatment in cystic fibrosis patients colonized by this bacterium.
- The aim of this project is to design a phage cocktail based in several lytic and non-transductant phages encoding an alginate lyase, and evaluate its safety and effectiveness *in vitro* and *in vivo*.

EXPERIMENTAL DESIGN

1· The **Step-by-step method (SBS)** will be used for the selection of infective phages against each resistant strain that could appear. The method ends when last phage-resistant mutant is sensible to the first phage [3].

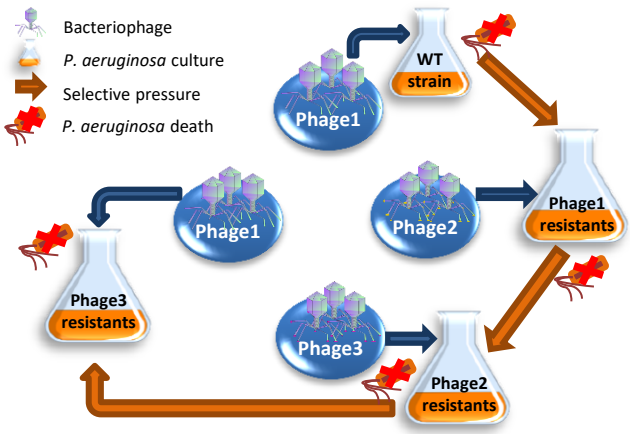

Figure1. Representation of the SBS method.

2· **Bacteriophage Recombineering of Electroporated DNA (BRED)** will be conducted for gene insertion in the phage genome. The genome and the desired gene are co-electroporated in a strain with a recombination system [4].

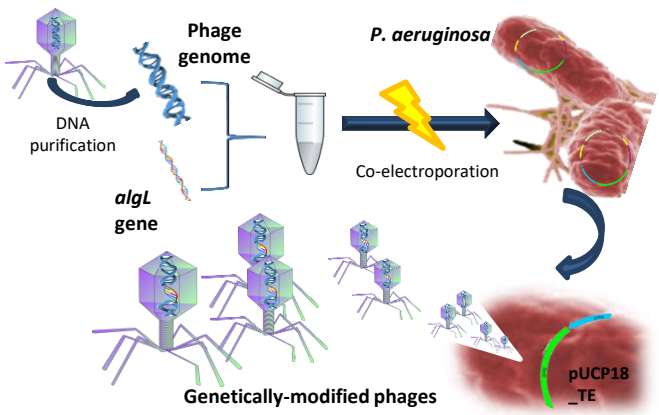

Figure2. Representation of BRED Technology.

3· *In vitro* **biofilm and cell viability analysis** will be performed in microtiter plates and continuous flow cells. Total biomass quantification and cell viability will be determined by Syto9 and FDA assays, respectively.

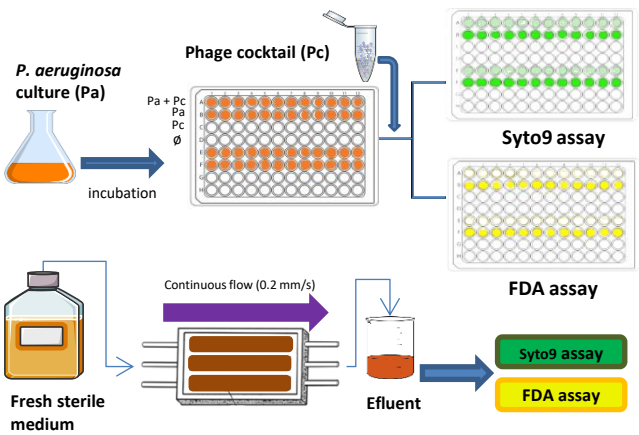

Figure3. Diagram of *in vitro* analysis of early (up) and mature (down) biofilms.

4· For *in vivo* determination of safety and effectiveness, survival rate of mice will be determined as well as quantification of cytokines, LDH, phages and bacteria in bronchoalveolar fluids.

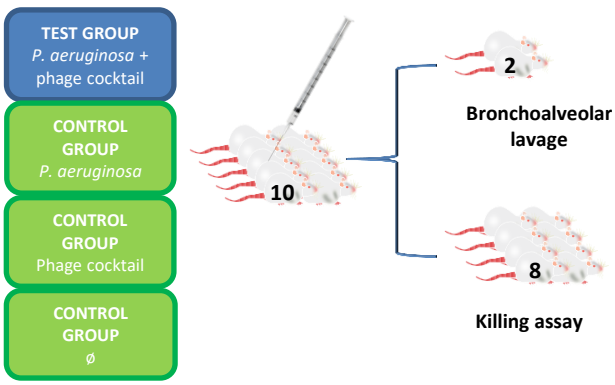

Figure4. Diagram of *in vivo* analysis and treatments.

EXPECTED RESULTS

- Reduction in viable cell numbers and total biomass in both early and mature biofilms.
- Significant increase in mice survival in the infected and treated group when compared with non-treated group.
- Cytokine and lactate dehydrogenase levels should be reduced, as well as bacteriophage and viable cell counts, when compared with non-treated group.

REFERENCES

1· Bodey, G. P., Bolivar, R., Fainstein, V., & Jadeja, L. (1983). Infections caused by *Pseudomonas aeruginosa*. Review of Infectious Diseases, 5(2), 279-313.

2· American Thoracic Society & Infectious Diseases Society of America. (2005). Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. American journal of respiratory and critical care medicine, 171(4), 388.

3· Gu, J., Liu, X., Li, Y., Han, W., Lei, L., Yang, Y., & Sun, C. (2012). A method for generation phage cocktail with great therapeutic potential. PLoS One, 7(3), e31698.

4. Marinelli, L. J., Piuri, M., Swigoňová, Z., Balachandran, A., Oldfield, L. M., van Kessel, J. C., & Hatfull, G. F. (2008). BRED: a simple and powerful tool for constructing mutant and recombinant bacteriophage genomes. PLoS One, 3(12), e3957.